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BIOLOGICAL BULLETIN.

FORM REGULATION IN CERIANTHUS.

I. THE TYPICAL COURSE OF REGENERATION.

C. M. CHILD.

INTRODUCTION.

During the year 1902-1903 it was my privilege to spend several months at the Zoölogical Station in Naples, as holder of the Smithsonian table. I take this opportunity to express my great indebtedness both to the Smithsonian Institution for the grant and to Professor Dohrn and all other members of the staff of the Zoölogical Station. A part of my time at Naples was devoted to the study of regeneration and other regulative processes in the Cerianthidæ, and an account of these observations and experiments is begun in the present paper.

So far as I am aware the only work upon regulation in *Cerianthus* is that of Loeb.¹ A review of this work is unnecessary at this time since the various points will be discussed in connection with my own observations as occasion arises.

My observations and experiments upon the Cerianthidæ fall into a number of groups, and, since they are somewhat extended, the account of the subject will be divided in a corresponding manner. In the present paper the usual "normal" course of regeneration resulting in a perfect animal is described. Later the problem of experimental control of regulation will be taken up, then variation and abnormalities in regulation and the factors concerned in their production.

THE NORMAL ANIMAL.

It is necessary to call attention to a number of the features of the normal anatomy and habits before proceeding to the description of the regenerative phenomena.

¹ Loeb, J., "Untersuchungen zur Physiologischen Morphologie der Thiere," I., Wurzburg, 1891.

Cerianthus solitarius, the species which formed the subject of most of the experiments, is considerably smaller than *C. membranaceus*. Owing to the varying degrees of distension and contraction accurate measurements of the form are difficult to obtain. A considerable number of specimens were measured when apparently fully extended and the body distended with water. These were all among the larger specimens, for the smaller individuals were discarded in nearly all cases. These measurements are of course only approximate and serve merely to indicate the general proportions of the specimens used for experiment. Under other conditions of contraction or distension these same individuals possess very different proportions. In all cases a single individual was measured repeatedly at intervals and the maximum measurements taken as representing complete extension. The following table presents a few such measurements of different individuals, the measurements being given in millimeters:

Length of Body.	Length of Marginal Tentacles.	Length of Labial Tentacles.	Diameter of Disc.	Diameter of Body in Oesophageal Region.	Diameter of Body Near Aboral End.
90	30-35	12-15	12	7	7
95	25-30	12-15	12	7	5
60	20	9-12	10	6	4.5

The specimens used were between these limits of size. A comparison of the measurements of the three individuals shows that the smaller specimen possesses different proportions from the larger, *i. e.*, its transverse diameters are relatively greater as compared with the length than those of the larger specimens. In other words, after the individuals reach a certain size further increase is chiefly an increase in length. Without giving the figures at this time to prove this point, since it will be taken up later in connection with the discussion of morphallaxis, it may be said that this difference in proportion between small and large specimens is of general and probably universal occurrence in *Cerianthus*. Smaller specimens are always relatively thicker than large ones.

In general form the body is nearly cylindrical, expanding orally to form the disc and tapering slightly posteriorly. At the aboral end is a small pore which under certain conditions permits

the exit of water. In the expanded condition the disc possesses the form of a broad shallow funnel extending from the base of the marginal tentacles to the margin of the mouth and continued aborally in the oesophagus. The mouth is slit-like in form with one siphonoglyph or gonidial groove at one end of the slit. The disc is marked with radiating lines, slightly depressed, which correspond to the lines of attachment of the mesenteries beneath the surface: these continue aborally in the oesophagus. The oesophagus extends aborally from the disc about $\frac{1}{6}$ – $\frac{1}{5}$ the length of the body when the animal is fully extended.

The marginal tentacles, as their name implies, are borne upon the margin of the disc, usually in about three rows, the number varying in grown specimens from about 41 to 71. About the margins of the mouth are the shorter labial tentacles which are fewer in number than the marginal tentacles, and form only a single circle.

The body appears brownish in color, but upon close examination is found to be marked with light longitudinal stripes or lines of varying width, some of which extend the whole length of the body while others are shorter. These are in reality merely unpigmented areas between the stripes of brown pigment. The color of the marginal tentacles is in general effect lighter than that of the body, but they are marked by transverse bands of dark pigment. The labial tentacles are brownish and usually unstriped. The disc and oesophagus in large, apparently old specimens are dark brown without definite striping.

As regards the internal anatomy certain points are of interest in this connection. It has long been known that the arrangement of the mesenteries in the Cerianthidæ differs in some respects from that in the other Actinozoa. In the oesophageal region all mesenteries extend from the body-wall to the oesophagus and thus divide the enteron of this region into a series of longitudinal radiating chambers which open into the enteron aborally. At the oral end each of these intermesenterial chambers opens into the cavity of a single marginal tentacle; thus the marginal tentacles are always equal in number to the intermesenterial chambers. The labial tentacles, while corresponding in position to intermesenterial chambers, are fewer in number.

Aboral to the oesophagus the inner margins of the mesenteries hang free in the enteron and bear the mesenterial filaments. A single pair of very short mesenteries at that end of the mouth where the siphonoglyph is situated are known as the directives. The next mesentery on each side of these extends almost to the aboral end of the body. From this point to right and left the mesenteries decrease in length, following a definite, rather complex law which need not be discussed here. On the side opposite the directives, at the opposite angle of the mouth are the shortest mesenteries, with the exception of the directives; these do not extend far aboral to the oesophagus. It is in this region that all new mesenteries are added, *i. e.*, the region of growth is opposite the directives. Thus, proceeding from the directives to the right and left around the body the mesenteries are successively younger. Each pair of new mesenteries appears between the members of the last preceding pair formed, thus separating them. Corresponding to the formation of new intermesenterial chambers new tentacles appear in this region. It is usually possible to find at this point in the normal animal one or two pairs of tentacles much smaller than the others and in process of growth. Corresponding to the number and arrangement of the mesenteries there is one unpaired marginal tentacle over the chamber between the directive mesenteries and known as the directive tentacle. It is usually somewhat thicker than the other tentacles since the space between the directives is greater than that between other mesenteries. The other tentacles are paired right and left.

In *Cerianthus solitarius* the greater number of the mesenteries about the whole circumference of the body do not extend aborally far beyond the oesophagus. Only certain mesenteries extend further, to end at various levels according to their position. This is also true of *Cerianthus membranaceus*.

The muscles of the body-wall consist of a heavy layer of powerful longitudinal muscles which decreases slightly in thickness toward the aboral end. These are the chief muscles of the body, circular muscles being absent, and tentacles, disc and oesophagus possessing only a slight muscular development.

As is well known, the Cerianthidæ are found imbedded in the

sand with the oral end and tentacles protruding. In this position they secrete about the body a mass of tenacious slime in which sand-grains and other foreign bodies become imbedded, the whole forming a tube into which the animal may withdraw. Loeb has given an interesting account of the geotactic reactions of these animals and my incidental observations upon this point confirm his. He has also described a number of experiments concerning the external conditions which determine the tube-formation.

When specimens are kept in aquaria without sand they creep about to a considerable extent, often climbing the sides. When left undisturbed they usually orient themselves as Loeb has noted, so that the oral end of the body is directed upward, even if this position necessitates the bending of the body at right angles. In the jars they secrete a considerable amount of slime and often form tubes along the sides or bottom, in which they remain for a longer or shorter time. When handled or otherwise irritated, and especially when cut, the secretion of the slime is especially rapid.

When undisturbed, the body and tentacles are usually more or less distended with water and the body-wall is always tense. Indeed, as will be shown later, complete extension of the body and erection of the tentacles is impossible without internal water-pressure, *i. e.*, without water in the enteron. If the body of a distended animal is opened quickly by a small cut the water issues with considerable force, and when an individual is made to contract rapidly by sudden stimulation the water squirts from the aboral pore with great force. The inability of the animal to extend to its full length without the aid of water-pressure is due to the absence of circular muscles in the body-wall. Extension is passive, not active. The longitudinal muscles are powerful and under strong stimulus the body may be torn apart if the ends are fastened.

It was found that the animals could be kept alive for months without other food than the small forms and organic particles which the water might contain, and in the present series of experiments no attempt was made to give them food. Of course in the early stages of regeneration and throughout many of the experiments the pieces were unable to take food; moreover, the

growth resulting from an abundant food supply constitutes in any case a complicating factor in the analysis of various phenomena of form regulation. In such experiments as permit the taking of food a complete analysis of the phenomena would include studies of the effect of abundant food supply, but previous experiments along this line indicate that the results in the lower animals differ only in degree and not essentially in kind with the presence or absence of food.

Four species of *Cerianthus* were employed for experiment, viz., *C. solitarius*, *C. membranaceus* and two smaller undetermined species, one of them almost completely colorless. It was soon discovered, however, that *C. solitarius*, a very common form in the Bay of Naples, was more favorable than the other forms on account of size, coloration of body and abundance. My attention was therefore devoted chiefly to this species, though the other forms, and especially *C. membranaceus*, were used for comparative study.

THE COURSE OF REGENERATION.

The cut pieces were isolated in dishes which were placed in aquaria supplied with flowing water. During the earlier stages of regeneration the pieces showed little tendency to creep out of dishes, but later it was necessary in some cases to cover the dishes with netting to prevent escape.

In *Cerianthus* the course of regeneration is complicated in many cases by various factors, such as the form of the pieces, the internal water pressure, etc. The simplest cases are those in which the body is divided by a transverse cut into two pieces, or a piece is removed by two transverse cuts. In such cases a nearly cylindrical piece is obtained which regenerates at the cut end or ends. Such pieces are best fitted for the study of the "typical" course of regeneration at the two ends, and since a knowledge of this is important as a preliminary to the study of experimental control of regeneration this paper is devoted to a description of the phenomena concerned in such cases.

A piece cut from the middle region of the body (*e. g.*, between the lines *aa* and *bb*, Fig. 1) will serve as an example.

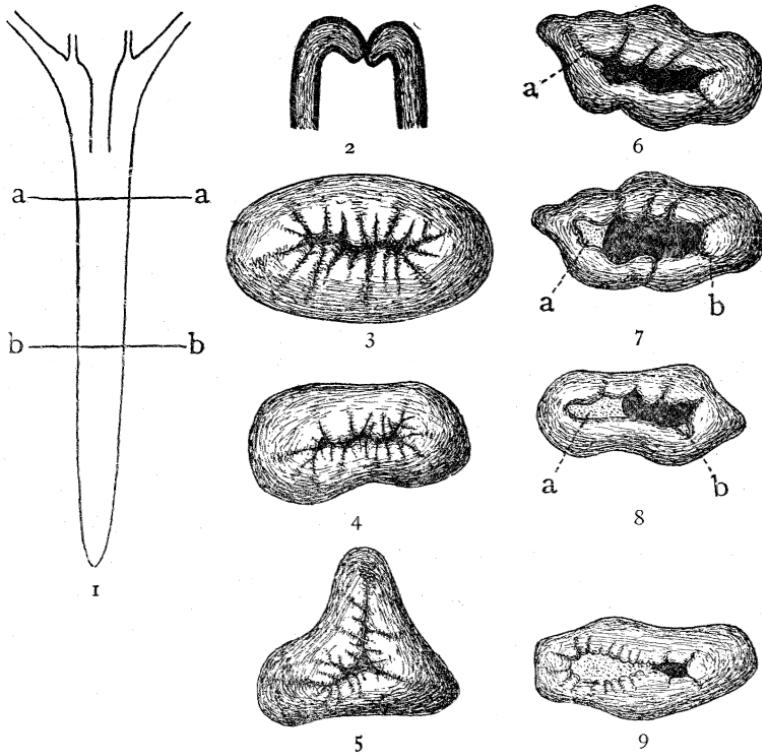
THE IMMEDIATE EFFECTS OF THE OPERATION.

Individuals which were in good condition and well extended were chosen and the cuts were made rapidly with sharp scissors. All parts of the body contract strongly in consequence of the cut, and of course total collapse of the piece occurs, owing to the escape of the water from the enteric cavity. Within a few moments the piece may relax somewhat from the extreme condition of contraction, but does not attain anything like its original length. Placed in the jar it lies on the bottom, and the weight of the tissues causes it to become more or less flattened. The piece has no power to retain its cylindrical form, though the mesenteries and mesenterial filaments, especially in pieces cut from the oral half of the body, partly fill the enteron and so cause the piece to retain a more or less rounded form. The body-wall is opaque in these pieces, while in normal specimens distended with water it is slightly translucent. The opacity is due simply to its greater thickness in the absence of the tension caused by internal water-pressure.

Within a few moments after section the cut edges at the two ends of the piece begin to bend or roll inward, and in an hour or two this inrolling has proceeded so far that the cut edges are no longer visible from the ends and the opening is almost completely closed by the inrolled portions. In Fig. 2 a longitudinal section through the oral end of such a piece is shown, the ectoderm and entoderm being indicated by full black lines and the thick muscular layer by fine lines. In this and following figures of the same kind the mesenteries are not shown; they of course occupy practically the whole of the enteric cavity after collapse. A section through the aboral end shows conditions similar to those figured in Fig. 2.

In consequence of the infolding about the whole circumference of the cut ends the circumference of the body-wall in the infolded region decreases greatly, although the transverse contraction of the body-wall during the infolding is not marked. It is, therefore, thrown into numerous longitudinal folds and ridges at the edge, and these appear when the piece is viewed from the end as folds and ridges radiating from what remains of the central opening.

Fig. 3¹ shows the oral end of a collapsed piece in which infolding has occurred. The numerous radiating foldings of the body wall are evident. Figs. 4 and 5 show the aboral ends of similar pieces. By this unfolding of the cut edges the openings at the ends of the piece are reduced to slits as is seen from



the figures, and various parts of the circumference of the cut edge are approximated, though actual contact between parts of the cut edge cannot occur everywhere, owing to the irregular wrinkling of the margin as it folds inward. Indeed, since the margin does not contract transversely to any great extent as the infolding occurs, actual contact of all parts of the cut edge is a physical impossibility, as it could occur only by the reduction of the cut margin to a point at the center of the circle formed

¹ In Figs. 3, 4, 5, 6, 7, 8, 9, 10, 12 the longitudinal pigmentation of the body is not shown. The new tissue, where present, is indicated by stippling.

by the body-wall. In most cases, however, as the collapsed, more or less flattened piece lies on the bottom of the jar the infolding edges come into contact along the longer margins as in Figs. 3 and 4, leaving an elongated slit between them. In other cases the closure may occur as shown in Fig. 5. In general the form of the end depends wholly upon physical conditions and especially on the form of the transverse section of the piece after collapse.

The infolding of the cut margins is undoubtedly the result of mechanical conditions, though these conditions may themselves be in part reactive in nature. As Loeb has pointed out, an infolding must occur if the inner portions of the body-wall are under greater longitudinal tension than the outer portions. Such a condition may possibly be produced in the muscles near the cut, the inner layers undergoing greater contraction than the outer, but the elasticity of the fibrillar mesogloea is probably in part responsible. As will be shown later this infolding produces in many cases conditions from which a return to the normal form is impossible. It can scarcely be regarded, therefore, as an adaptive reaction in the stricter sense. The radiating wrinkles and folds upon the end are due simply to the fact that the cut edges do not contract transversely as they are folded in.

As already noted, the result of the infolding is to close the terminal openings more or less completely. The closure is in no case perfect since between the irregular wrinkles there are always numerous interstices which afford communication between the enteron and the exterior. In most cases, however, these are soon blocked by the tenacious slime secreted by the ectoderm and are also frequently more or less completely filled by portions of the mesenteries or the filaments which happen to extend through them from within.

THE CLOSURE OF THE ENDS.

The histological changes about the cut margins have not been fully investigated as yet, but it has been determined that growth of new tissue upon the edges begins soon after the cut is made. If after one or two days the infolded end be opened and carefully spread apart a very thin and delicate whitish membrane of new

tissue will be found extending across parts of the opening. While growth undoubtedly begins on all parts of the cut surface, this membrane becomes distinct earlier at those regions where the cut edges are most closely approximated. Frequently when a piece is opened in the manner described the membrane will be found extending across regions corresponding to certain of the wrinkles about the opening but not yet covering the central area.

This method of formation of the thin membrane closing the end is well shown in a piece cut from a specimen of *C. membranaceus*. In this species the body-wall is so thick and stiff and the diameter of the body so great that in short pieces the infolding of the ends is often not sufficient to close the opening. In Fig. 6 a piece of this kind is shown. The new tissue first became evident along the fold *a*, and a day or two later a thin membrane was spread across this fold (Fig. 7, *a*. The new tissue is stippled). A little later still the fold at *b* (Fig. 7) also showed a thin membrane (Fig. 8), which, however, was afterward ruptured by contractions of the piece due to the stimulation incidental to examination. In Fig. 8 it is seen that the new tissue is gradually spreading over the opening from *a*. In Fig. 9 the opening is nearly closed. Several days later closure was complete. The changes in form of the piece as shown in the figures were the result of stimulation caused by the manipulation necessary for examination and drawing. In *C. solitarius* if the pieces are allowed to remain undisturbed at ordinary summer temperature the openings at the ends are usually completely closed by the thin membrane on the third day after operation. In the piece from *C. membranaceus* above described closure was complete after twenty-seven days. In general this species regenerates much more slowly than *C. solitarius*, but here the closure was exceptionally slow.

The membrane is easily ruptured by the contractions of the piece when strongly stimulated and great care is always necessary in the examination of such pieces to prevent rupture. In consequence of contraction the different parts of the margin change their relative positions or the mass of the mesenteries and filaments exerts pressure from within, thus readily causing rupture.

There is little difference as regards time of closure between the

two ends, though in general the oral end is slightly in advance of the aboral end.

DISTENSION OF THE PIECES WITH WATER.

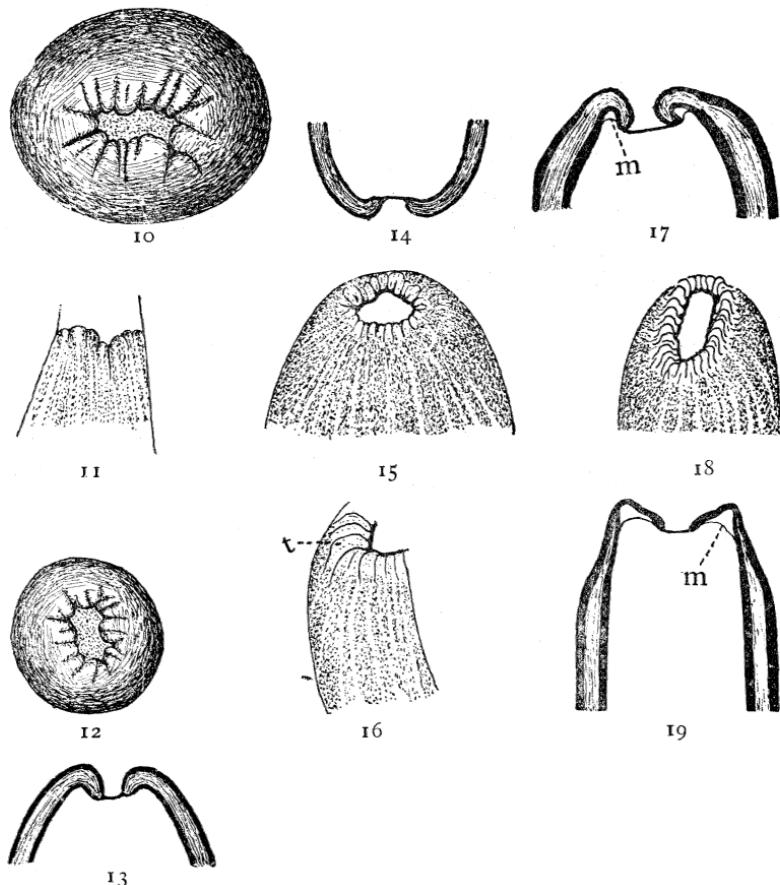
The piece remains completely collapsed during two or three days in summer and five to six days in winter, and then gradually becomes distended. At this time the piece is completely closed at both ends, no mouth or aboral pore being present. It is probable that the accumulation of water in the enteron is the result of diffusion through the walls, and especially through the very thin membranes at the two ends, in consequence of the accumulation of soluble products of metabolism in the closed enteron.

In the course of a day or two the piece becomes well filled with water and attains a degree of distension approaching that of the normal animal, though not as great. In some cases the accumulation of water in the enteron occurs so rapidly that the thin membranes closing the ends are ruptured and collapse occurs again, though usually the increase in thickness and strength of the membrane is sufficient to prevent rupture. The piece is usually well filled with water by the fourth day in summer and usually by the seventh or eighth in winter.

The immediate result of the renewed distension of the piece with water is, of course, the resumption of the cylindrical form; the body wall becomes translucent and is elastic to the touch like that of the normal animal.

The most marked effect of the internal water pressure occurs, however, at the ends of the piece. So long as the piece remains collapsed the thin membrane closing the ends is not visible since the infolded edges of the body-wall are in close contact. As the body becomes distended with water, however, the infolded portions gradually spread apart and a central area covered by the new tissue becomes visible. Very small at first, it gradually increases in size until its diameter is about one third the diameter of the end. Fig. 10 shows the oral end of a piece at about this stage. The area within the folded margin of the old body-wall is covered by the thin membrane of new tissue. In Fig. 12 the aboral end of a similar piece is shown. There is

little difference between the two ends, except that growth of the membrane is more rapid at the oral end. In Fig. 11¹ a portion of the oral end is shown more highly magnified. In this case the abrupt transition from the pigmented body-wall to the almost colorless new tissues is evident. From this figure it is also seen that the margin of the old body-wall is somewhat crenated by



fine folds and wrinkles, which, however, are not regular in size and form, and do not represent the early stages of the new tentacles. The slight folds indicate more or less exactly the regions where the mesenteries are attached and the bulging areas

¹ In Figs. 11, 15, 16, 18 the pigmentation of the body-wall is indicated.

between the intermesenterial chambers, these being now filled with water and under pressure. Here and there, however, folds without such significance occur, and moreover some of the chambers are so situated on the infolded margin that they are more widely open and thus expand more in consequence of the pressure than others, hence the irregularity in form and size of these crenations.

In Figs. 13 and 14 are shown respectively the oral and aboral ends of the body-wall at the stage where the infolded portions begin to separate. The thin membrane closing the end is shown as a black line. It consists, of course, of ectoderm and entoderm, but the muscular layer does not extend into it.

THE FORMATION OF THE MARGINAL TENTACLES AND DISC.

Within the first day or two following the closure of the ends and the distension of the piece with water the changes leading to the formation of the characteristic organs of the oral end begin. In pieces cut from the middle region of the body the full number of mesenteries is not present, since some end anterior to this region. Regeneration of mesenteries occurs, though the number of mesenteries in a regenerated oral end from the middle region of the body is somewhat less than the number originally present at the oral end of the individual from which the piece is taken. This point will be considered at another time. It is sufficient for the present purpose to say that the whole oral end of the piece becomes divided into intermesenterial chambers, in the manner characteristic of the species, by the regeneration of new mesenteries, at first very short, between the longer mesenteries which are present in the piece. Attention was called above to the crenation of the infolded margin in correspondence with the position of the mesenteries (Fig. 11).

The first marked change following the closure of the end is the appearance of a slight ridge on the infolded margin of the old body-wall as shown in Fig. 15. The ridge is wholly confined to the tissue of the original body-wall, the thin membrane which closes the end playing no part in its formation. The crenations become more distinct and extend in many cases from the margin of the old body-wall over the ridge, as the regeneration of the

mesenteries beneath advances. In Fig. 15 the ridge is shown as slightly lighter in color than the rest of the body. The pigmentation is beginning to disappear. Most of the stripes can still be followed over the ridge to the margin of the old tissue, but upon the ridge they are fainter than before. Fig. 16 shows a portion of the end at a slightly later stage, more highly magnified. Here the lighter color of the ridge is more distinct. While the body in general retains its brown color the ridge becomes light yellowish and its pigment disappears completely in the course of a day or two.

This change in pigmentation indicates that some alteration in the tissues is occurring, and the nature of the alteration becomes evident when a longitudinal section through the end (Fig. 17) is examined. This figure shows that the thickness of the body-wall and especially of the muscular layer is decreasing considerably in the region corresponding to the ridge. This decrease is shared to a certain extent by the ectoderm and entoderm as the figure indicates. The new regenerating mesenteries are minute folds in the infolded region, ending free aborally (*m*, Fig. 17).

This ridge in which loss of pigmentation and reduction in thickness of the body-wall are taking place may be designated as the *marginal tentacular ridge*, since it is from this that the marginal tentacles arise; indeed the reduction in thickness of the body-wall and the division of the ridge into areas corresponding to the intermesenterial chambers are the preliminaries of tentacle formation.

The marginal tentacles do not arise from the cut edge of the body-wall itself but a short distance away from it, *viz.*, at the highest point of the ridge (*t*, Fig. 16), *i. e.*, entirely within that portion which was originally part of the body-wall and not in the new tissue which closes the end.

Fig. 18 shows the oral end of a piece about a day later than the stage shown in Figs. 15 and 16. Here the new marginal tentacles are distinct and are evidently increasing in length. The pigment has disappeared completely from the tentacular ridge which is now whitish in color and distinctly translucent. Some of the tentacle buds are slightly broader than others owing to the fact that in the infolded condition of the margin some inter-

mesenterial chambers were compressed and others stretched according to their position on the folds. There is, however, no marked difference in the length of the new tentacles on the different sides of the body, those in the region of the directives being no more advanced than those in the growing region opposite. From this figure it is very evident that the marginal tentacles arise from the highest, *i. e.*, the most oral point of the tentacular ridge. Moreover they arise in a single circle or row, although in the normal animal they occur in about three concentric circles.

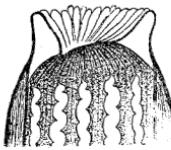
A longitudinal section of the body-wall at this stage is shown in Fig. 19. A comparison with Fig. 18 shows marked changes. The most conspicuous of these is the continued reduction in thickness of the body-wall upon the ridge. The muscular layer has almost or quite disappeared in this region and also between it and the new tissue occupying the central region of the end, and is reduced considerably in thickness for some distance aboral to the ridge. At this stage then the whole oral end is closed by a thin, unpigmented, translucent membrane consisting of ectoderm and entoderm, but without a distinct muscular layer. The central part of this membrane resulted from the growth of new tissue at the cut edge, while the more distal portions forming the tentacular ridge have arisen by the transformation of a part of the old body-wall into tissue capable of a large amount of new growth, and of differentiation into new structures. In other words, the body-wall in this region has changed from its differentiated condition to what is commonly called the embryonic condition. The histological features of this change are of great interest, but will be described at another time.

The marginal tentacles now grow rapidly, and in another day (six days after the operation in summer) the oral end presents the appearance shown in Fig. 20. Several changes of importance have occurred since the stage of Fig. 18: the disc is greatly expanded, the marginal tentacles are much longer, the distinction between the tissue of the old body-wall and the thin membrane closing the end has disappeared completely, and finally the mouth is beginning to appear as an opening between the center and the periphery of the disc in the directive radius. The disc is

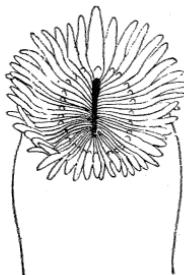
marked with radiating lines, each of which terminates distally between the bases of two tentacles; those lines are in reality grooves and mark the lines of attachment of the mesenteries to the aboral surface of the disc. It will be seen that a small area



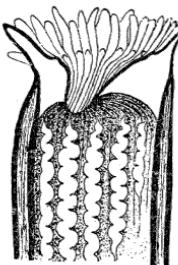
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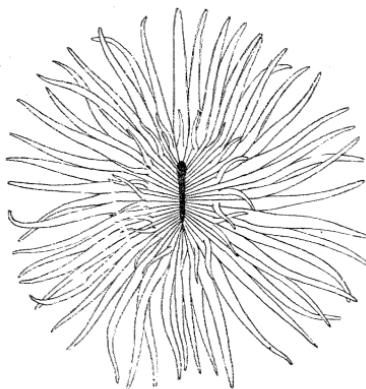
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in the center of the disc, indicated in the figure by stippling, is free from these lines; this represents that portion of the thin membrane beneath which regeneration of the mesenteries has not yet occurred. In the directive radius is situated a small opening, the new mouth, which gradually elongates in the directive plane.

The directive tentacle, which corresponds to the chamber between the two directive mesenteries, is slightly thicker than the other tentacles in consequence of the fact that the directive mesenteries are somewhat farther apart than other mesenteries. As regards the arrangement of the marginal tentacles it will be seen that they are no longer in a single row, but some appear as if they were being crowded out of the row, owing to lack of space. In all probability that is what is occurring. As the tentacles increase in size there is not sufficient space for them in a single row upon the margin and some are pushed out, probably in most cases peripherally.

Fig. 21 is a schematic figure of one half the body after longitudinal section in the directive plane, the directive tentacle being on the left of the figure. The stage of regeneration is about the same as that of Fig. 20. As compared with the earlier stages (*e. g.*, Fig. 19) several points of difference are to be noted: the marginal tentacles are longer, the difference in thickness between the reduced body wall of the tentacular region and the thin new tissue across the disc has completely disappeared; the reduction and disappearance of the muscular layer extends further aborally than before; the regeneration of the mesenteries has advanced; and finally there is a minute mouth, which, as was evident from Fig. 20, is not centrally placed, but lies near the base of the directive tentacle.

One or two points of importance as regards the regeneration of the mesenteries may be noted. In the normal animal a slight furrow, which appears as a faint longitudinal line on the surface of the entoderm, extends aborally from the aboral end of each mesentery. In a piece cut from the middle region many of the mesenteries lie wholly oral to the cut and so are not present in the piece, but most of the furrows, extending aborally, are visible in the piece. The mesenteries regenerate along these furrows. Whether regeneration of a particular mesentery aboral to the end of the furrow representing that mesentery is possible has not yet been determined. The point to which it is desired to call attention here is that the mesenterial regions are determined for some distance posterior to each of the mesenteries themselves.

Those mesenteries which extend into the piece undergo regressive changes, losing their thickened margins and filaments at the oral end, and become united with the new oesophagus.

THE APPEARANCE OF THE LABIAL TENTACLES AND THE LATER STAGES OF ORAL REGENERATION.

The marginal tentacles continue to increase rapidly in length and the oesophagus extends further across the disc from the directive side and also becomes deeper.

Fig. 22 is drawn from a stage three days later than Fig. 20 (nine days after the operation). Comparison of this figure with Fig. 20 shows at once the increased diameter of the disc, the greater length of the tentacles, and the marked change in the size and shape of the mouth opening. The tentacles in Fig. 22 are still of about equal size and length, except the directive tentacle, which is somewhat thicker and longer than the others. They still retain, to a large extent, the arrangement in a single row, though here and there a few have been forced out of line.

Upon the disc and forming a circle about the mouth appear the earliest stages of the labial tentacles. They are at this time mere buds, less than one half millimeter in length. All appear nearly simultaneously and develop with equal rapidity. As noted above, they are fewer in number than the marginal tentacles, some of the intermesenterial chambers possessing none.

A view of half the oral end at the stage of Fig. 22 after longitudinal section in the directive plane is shown in the schematic Fig. 23. In this case the plane of section passed through one of two small tentacles in the growing region opposite the directive tentacle; the section of this tentacle (on the right of the figure) is thus considerably smaller than that of the directive tentacle opposite. Comparison of Figs. 21 and 23 shows the changes which have occurred during the three days elapsing between the two stages. The cesophageal invagination is much deeper, the opening to the enteron is larger and the area of growing tissue, including the reduced body-wall, is much greater.

From this time on the course of regeneration consists in the gradual increase in size and the pigmentation of the regenerated parts in the manner characteristic of the species.

The problem of "morphallaxis," *i. e.*, the changes in the proportions of regenerating pieces leading to the more or less complete reestablishment of the "normal" form will be considered elsewhere.

Fig. 24 shows a regenerated disc and tentacles at a later stage; in form and general arrangement of parts it is not distinguishable from the normal animal. The marginal tentacles have not yet fully attained their final arrangement; at present they are in two fairly well marked rows or circles. During the still later stages, however, as further increase in size occurs, the bases of some are forced still farther peripherally and so the characteristic arrangement of tentacles is finally acquired. The pigmentation of the marginal tentacles with dark transverse bands, which appears at this stage or earlier, is not shown in the figure.

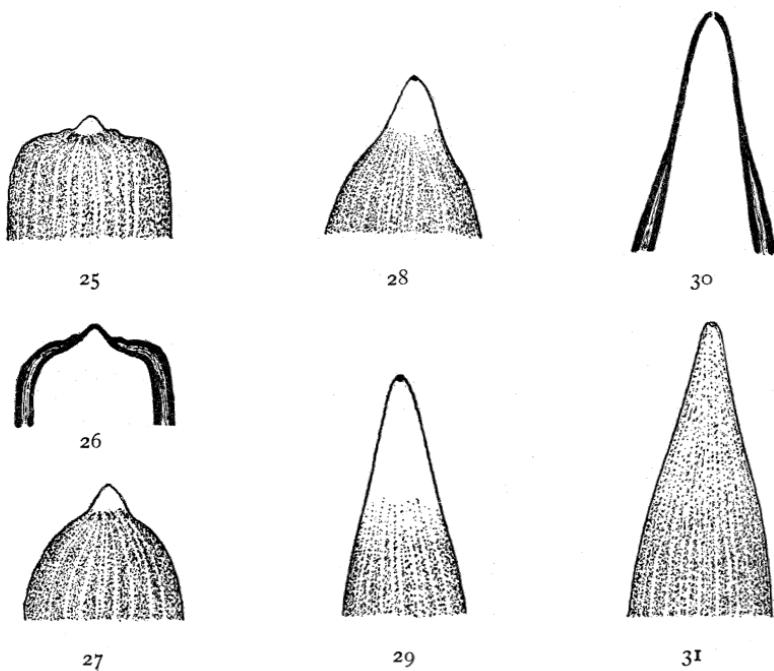
THE DIFFERENTIATION OF THE ABORAL END.

The infolding of the body-wall and the closure of the aboral end of a piece by a thin membrane have already been described. It remains to describe the formation of the characteristic aboral end. The course of regeneration here is much simpler than at the oral end.

The first marked change from the condition shown in Fig. 14 consists in the protrusion in conical form of the thin membrane closing the end (Fig. 25). About the margin of this new tissue the slightly wrinkled margins of the old body-wall are still clearly marked. In Fig. 26 a longitudinal section of the aboral end at this stage is shown. The absence of the aboral pore is to be noted. This new outgrowth at the aboral end does not become well-marked at once after closure, but only after the piece is well filled with water and the regeneration is advanced at the oral end, *i. e.*, it is much slower than oral regeneration.

In Fig. 27 the aboral outgrowth is seen at a somewhat more advanced stage. The wrinkles and folds upon the margin of the old tissue are gradually disappearing as this stretches and undergoes remoulding. A few days later the wrinkles have disappeared and there is no sharp distinction between the old body-wall and the new tissue at the time of union. Fig. 28 shows the end at this stage; and it is evident that the margins of the

old body-wall are becoming involved in the regenerative changes in the same manner as at the oral end, for the pigment stripes are gradually fading out in the region which was before infolded. Fig. 29 shows a still later stage in which the gradual fading of the pigment-stripes is clearly seen. The significance of this loss of pigment is made clear by Fig. 30, a longitudinal section of the aboral end at this stage. Here it is seen that a reduction of the muscular layer is occurring, *i. e.*, the old body-wall is becoming



involved in the regulative changes for a short distance oral to the cut end: In other words the new aboral end is formed not merely from the new tissue which closes the end soon after operation, but, as in the regeneration of the oral end, in part from tissue derived from the margins of the body-wall near to the cut surface, by reduction of the muscular layer and growth of the ectoderm and entoderm. Thus the distinction between "old tissue" and "new tissue," at first well-marked, gradually disappears in this region.

Fig. 31 shows a still later stage in which the new tissue is becoming pigmented. The appearance of the pigment corresponds in time with the differentiation of the muscular layer, and I am inclined to believe that in *Cerianthus* as in various other forms, the pigmentation of the body is closely connected with the presence and arrangement of the muscular layer.

The course of regeneration described in the present paper is characteristic of pieces cut from the middle half of the body. In following papers the regeneration of pieces from various regions will be compared, and experiments determining some of the factors concerned in regeneration will be described.

SUMMARY.

1. In cylindrical pieces of *Cerianthus* obtained by two transverse cuts collapse occurs at once and the cut ends begin to roll inward soon after section, finally coming into contact and closing the opening more or less completely. Since little or no transverse contraction of the infolded margins occurs they are thrown into numerous radiating folds and wrinkles.

2. Within two to three days after section a thin membrane formed by the growth of new tissue from the cut surfaces closes the two ends completely. The piece now becomes gradually distended with water, probably owing to the accumulation of metabolic products in the enteron and consequent diffusion of water into this closed cavity. As distension proceeds the infolded margins of the body-wall at the two ends are forced apart by internal pressure and the area occupied by the thin membrane increases.

3. The first step in the regeneration of tentacles is the formation of a slight ridge, the marginal tentacular ridge, on the oral end. This ridge is formed wholly within the tissue of the old body-wall, its formation being accompanied by reduction and disappearance of the muscular layer, disappearance of the pigment and great reduction in thickness. The marginal tentacles first appear as slight upgrowths from the highest—most oral—point of the ridge, one tentacle corresponding to each intermesenterial chamber. The position of the mesenteries is indicated externally on the tentacular ridge by slight furrows which separate the regenerating tentacles from each other.

4. The regenerating marginal tentacles appear at first in a single circle and all usually regenerate with nearly equal rapidity, except in some cases the youngest pair in the growing region. The directive tentacle is usually slightly thicker than the others since the directive mesenteries are somewhat farther apart than the other mesenteries. Rapid increase in length occurs in the marginal tentacles, and the arrangement in about three circles or rows is gradually attained in consequence of the fact that there is not sufficient space on the margin of the disc for all of the tentacles in a single row; some are forced peripherally by the mutual pressure exerted.

5. As the tentacles grow the disc expands and the distinction between the thin membrane of new tissue which first closed the end and the old body-wall with which it was connected disappears completely in consequence of the complete disappearance of the muscular layer, the reduction in thickness, and the loss of pigment in the body-wall of the oral end.

6. The mouth appears after the marginal tentacles are well established near the base of the directive tentacle, gradually extending along the directive plane across the center of the disc until it is symmetrical. The part of the mouth first regenerated is the region of the siphonoglyph.

7. The labial tentacles do not appear until the marginal tentacles have attained a length of several millimeters. Each tentacle appears as a distinct bud over an intermesenterial chamber, but some intermesenterial chambers are without labial tentacles.

8. After the aboral end is closed by the new tissue this slowly acquires a conical form, protruding from within the wrinkled margin of the old body-wall. The wrinkles on the latter gradually disappear and the pigmentation slowly fades out for a short distance oral to the cut end, this change being connected with reduction and disappearance of the muscular layer as this region of the body-wall becomes involved in processes of growth and redifferentiation in the same manner as the oral end. The aboral end grows out into an elongated conical form at the end of which the aboral pore appears. As the new muscles differentiate in this region pigment stripes begin to appear.